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Award Number: W81XWH-05-1-0308

TITLE: Chemoprevention against Breast Cancer with Genistein and Resveratrol

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REPORT DATE: March 2006

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-03-2006	Annual Summary	25 Feb 2005 – 24 Feb 2006

4. TITLE AND SUBTITLE

Chemoprevention against Breast Cancer with Genistein and Resveratrol

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0308

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Timothy, G. Whitsett, Jr.
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Alabama at Birmingham
Birmingham, AL 35294

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

Breast cancer remains a destructive disease despite new therapeutics. It is well accepted that environmental factors, especially diet, can play an important role in determining one's future risk of the disease. We believe that two natural polyphenols, genistein (a component of soy) and resveratrol (a component of grapes and red wine), can suppress mammary carcinogenesis. We and others have clearly shown a mammary-protective effect against chemically-induced mammary cancer. This project aims to elucidate mechanisms through which these polyphenols may exert their effects. We show that genistein and resveratrol can modulate the protein expression of several critical proteins in the mammary gland that are involved in growth and proliferation. We see changes in both the MAPK signaling pathway, the PI3K/Akt pathway, as well as changes in sex steroid receptor cofactors. Future aims will look at the importance of the estrogen receptors in the mechanisms of these polyphenols and determine whether these polyphenols can suppress cancer in a novel mouse model for breast cancer.

15. SUBJECT TERMS
Genistein, Resveratrol, Mammary Cancer, Chemoprevention

16. SECURITY CLASSIFICATION OF:				18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER *(include area code)*

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Introduction:

Breast cancer remains a destructive disease and a leading killer among women in the United States and throughout the world (1). It has been recognized that genetic alterations (such as BRCA mutations) account for only 10-15% of breast cancer. Thus, environmental exposures, especially diet, can play a very important role in the causation or prevention of this disease. We believe that the dietary polyphenols genistein, the major phytoestrogen in soy, and resveratrol, a component of red grapes and red wine, can protect a woman against mammary carcinogenesis. We, and others, have shown that dietary exposure to genistein or soy, especially early in life, can protect against chemically-induced carcinogenesis (2-3). We demonstrated that prepubertal exposure to genistein caused a significant reduction in terminal end buds, the most susceptible structures for mammary carcinogenesis. We and others have also shown a protection against breast cancer in a chemically-induced rat model with dietary exposure to resveratrol (data unpublished, 4-5). Resveratrol caused a significant reduction in mammary tumor multiplicity and increased tumor latency. The epithelial cells of the terminal end buds show a significant reduction in proliferation and increase in apoptosis, which might help to explain the chemoprotection that we observed. With observations from the tumorigenesis, mammary gland maturation, and cell proliferation experiments, we proposed to look for changes at the molecular level that could account for the protection we observe with dietary genistein and resveratrol. We propose to focus on steroid and growth factor receptor pathways, and the steroid receptor coactivator family, a possible link between critical sex steroid and growth factor pathways. Understanding the in vivo mechanisms of these polyphenols will allow them to be used to protect women against breast cancer.

Body:

To discuss the research accomplishments in the first year (Feb. 2005 – Feb. 2006), the original Statement of Work will be used with each of three aims being discussed.

Aim 1. (Months 1-12)
The goal of this aim was to investigate the potential of genistein and resveratrol, alone and in combination, to regulate critical sex steroid receptors, steroid receptor coactivators, and critical growth factors and receptors in the mammary glands of Sprague Dawley rats.

The 4th abdominal mammary glands have been dissected from both 21- and 50-day-old rats treated ± the polyphenols genistein and resveratrol, alone and in combination. Immunoblot analysis has been employed to look at the protein expression of several sex steroid receptors, steroid receptor coactivators, and growth factor receptors. The steroid receptor coactivator GRIP-1, was shown to be up-regulated at 21 days postpartum by genistein in the diet (data not shown). This is followed by a decrease in GRIP-1 expression at 50 days postpartum (Figure 1). This fits a model proposed by our lab that genistein causes an increase in mammary gland proliferation and maturation early in the animals, which later in life results in a more mature gland that is less proliferative

and thus less susceptible to carcinogenesis. More recently, we observed a decrease in the expression of SRC-1, another coactivator that enhances steroid receptor signaling, at 50 days postpartum. We did not observe significant differences in the sex steroid receptors such as the estrogen receptors alpha and beta or progesterone receptors at 21 or 50 days

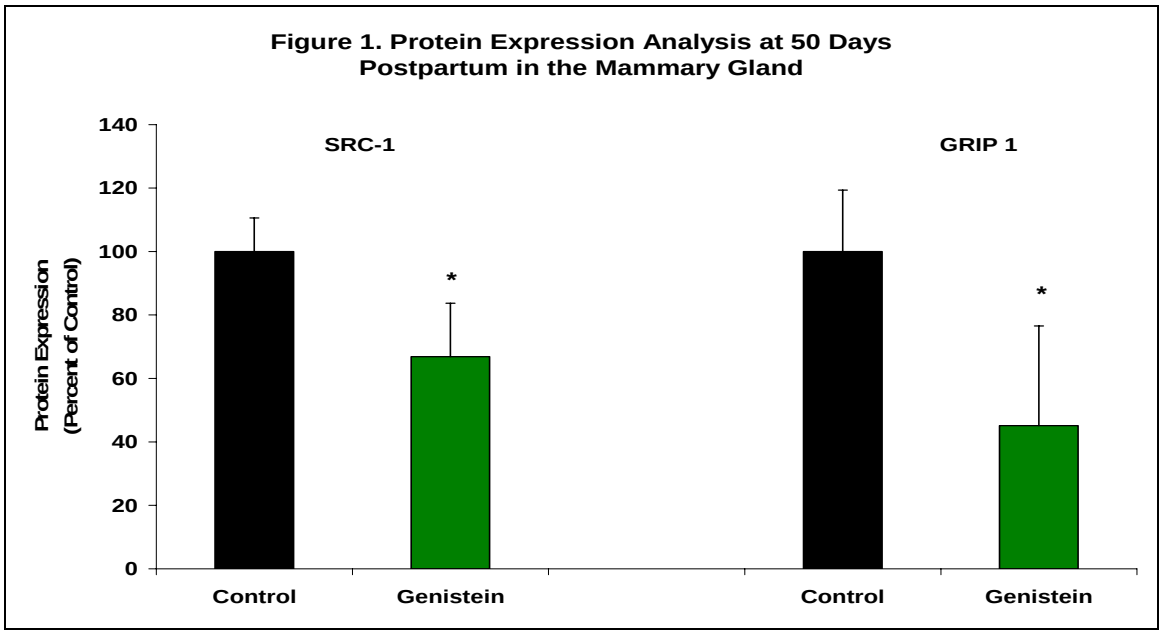

Figure 1: Rats treated ± genistein (250mg/kg diet) were sacrificed at 50 days postpartum. Mammary glands were dissected and assayed for protein expression by western blot. Results are given as means + SEM, with the Control set to 100. * represents a p value < 0.05

postpartum. Likewise, we did not see significant regulation of the epidermal growth factor receptor, insulin-like growth factor receptor, or total and phosphorylated ERK 1 and 2 at 21 or 50 days postpartum (data not shown).

Resveratrol-treatment also caused protein modulation which might help to explain the protection that we observed against mammary carcinogenesis. At 50 days postpartum, we observed a significant reduction in the level of GRIP-1, the same that we saw with genistein treatment. We also saw decreases in IGF-1R (insulin-like growth factor-1 receptor), Akt, and the phosphorylated (active) form of Akt. All of these have been implicated in mammary cell proliferation and carcinogenesis. A reduction in these growth factors may help to explain the mammary chemoprevention that we observed in the rat model. Again we observed no differences in the protein expression of the estrogen receptors or the progesterone receptors at 21 or 50 days postpartum. We saw no changes in the steroid coactivators at 21 days postpartum. At 50 days postpartum, there was a trend toward reduction in the phosphorylated forms of ERK 1 and 2, but it did not reach statistical significance (data not shown). Thus, resveratrol and genistein may exert chemoprotective actions on growth factor signaling molecules, and the coactivator molecules for the sex steroid receptors.

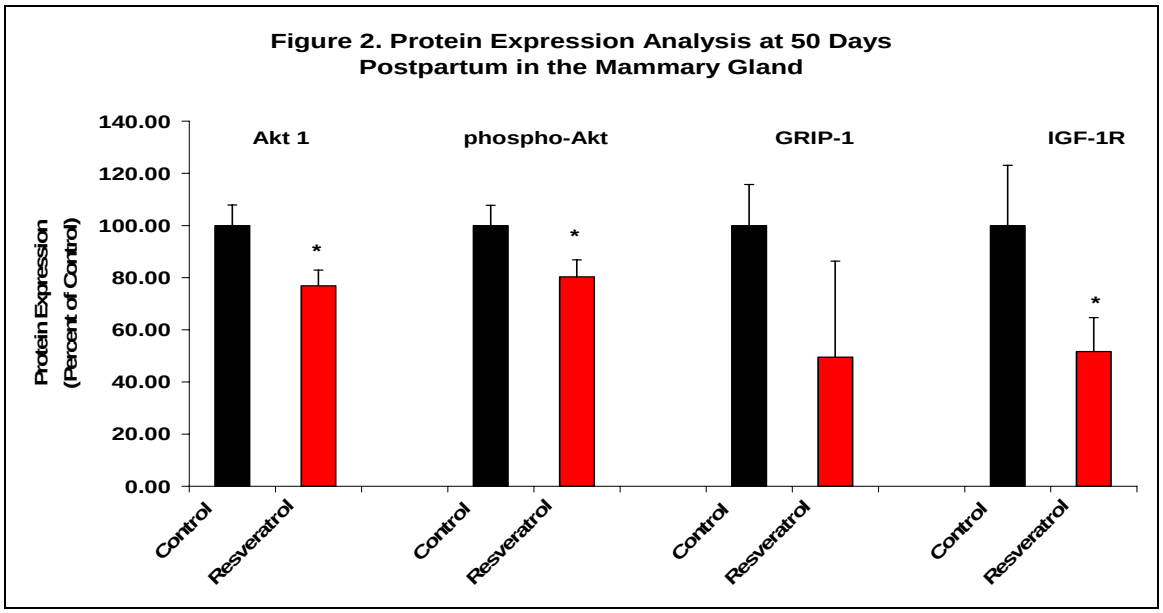

Figure 2: Rats treated ± resveratrol (1000mg/kg diet) were sacrificed at 50 days postpartum. Mammary glands were dissected and assayed for protein expression by western blot. Results are given as means + SEM, with the Control set to 100. * represents a p value < 0.05

The combination of genistein and resveratrol as well as the treatment with estradiol in the diet are currently under investigation at both 21 and 50 days postpartum.

Aim 2. (Months 12-24)
The goal of this aim was to investigate if the polyphenols, genistein and resveratrol, can act independent of the estrogen receptors. This will be accomplished by blocking both ER-alpha and beta using a pure antiestrogen, ICI 182,780, and looking at the same signaling molecules described in aim 1.

Although this aim is not supposed to start until year two of this project, we have already raised these animals, treated them, and sacrificed them. Thus, all of the glands have been collected for protein expression analysis, and this aim should be completed in the near future. We have employed the technique of bilateral ovariectomy, to remove ovarian estrogens from these rats, to better understand the importance of the estrogen receptors in polyphenol chemoprevention. The lack of background noise from endogenous estrogens should improve the results on the estrogenic mechanisms of these polyphenols.

Aim 3. (Months 1-36)
The goal of this aim is to investigate the potentials of genistein and resveratrol to suppress mammary tumorigenesis in a novel mouse model that over-expresses AIB1 (6).

The over-expression of human AIB1 in these mice is sufficient to cause tumorigenesis, although it takes time (up to 2 years) to develop these tumors.

Our colony of AIB1 transgenic mice has been expanded. We decided to run a detailed ontogeny study, looking at 4 mice every month to get a better understanding of the timing of tumorigenesis in this model. We have enough mice to sacrifice and evaluate 4 mice every month from month 4-24 postpartum. We have also started the groups for the tumorigenesis study. There are four groups: control, genistein, resveratrol, and a combination of genistein + resveratrol. Thus far, we have more than 30 mice per group and plan to add more these groups. These animals will begin to be palpated for tumors in the next couple of months. This aim is well on track and should yield exiting results.

Key Research Accomplishments:

- Significant modulation of several proteins was detected. Many of these are important for mammary growth, proliferation, and chemoprevention by genistein and resveratrol at 50 days postpartum.

- Produced, treated, and sacrificed all of the rats necessary for Aim 2, months ahead of schedule.

- Produced a colony of AIB1 transgenic mice from two breeder pairs big enough to run an ontogeny study and the proposed tumorigenesis study.

- Submitted a manuscript to the Journal of Carcinogenesis dealing with the chemopreventive properties of resveratrol.

- Data from this project was used to attend a conference and present a poster at the 2005 Gordon Research Conference: Hormonal Carcinogenesis. A graduate student fellow award was received for the work.

- Data from this project was used to attend and present a poster at the 2005 Society of Toxicology national meeting. A graduate student travel award was received for the work.

- Data from this project was used to attend and present a poster at the 2005 UAB Comprehensive Cancer Center 2005 Annual Research Retreat.

- Some of the data obtained from this project was used to help the PI (Tim Whitsett) qualify into candidacy in the UAB Pharm/Tox doctoral program.

- Data from this project was accepted for poster presentation for two separate meetings in 2006: AACR annual meeting and the Gordon Research Conference: Mammary Gland Biology.

Reportable Outcomes:

Some of the results from this grant have been used in poster presentations at national scientific meetings. In 2005, two meetings were attended with data from this grant. The abstracts are as follows:

1) Whitsett T and Lamartiniere CA. Breast Cancer Chemoprevention with the Polyphenol Resveratrol. Gordon Research Conference: Hormone Action in Development and Cancer. July 2005.

A graduate student fellow award was received for the work.

2) Whitsett T and Lamartiniere CA. Breast cancer chemoprevention with the polyphenol resveratrol. Society of Toxicology 44 Annual Meeting. Program page 164. March 2005.

A student travel award was received for the work.

The data was also used in an invited seminar at UAB. The title of the seminar was: "Mammary Cancer Chemoprevention with the Polyphenols Genistein and Resveratrol" UAB Pharmacology and Toxicology Seminar, Feb. 2006

Conclusions:

We and others have clearly shown a protection against mammary carcinogenesis using the polyphenols genistein and resveratrol in the diet. We have also shown the ability of these polyphenols to modulate mammary gland maturation, as well as cell proliferation and apoptosis. This grant aims to look even deeper, at the molecular level to elucidate the mechanisms through which these polyphenols act. Through year one of the project, we have shown that genistein and resveratrol can regulate important molecules in both the growth receptor pathways and the sex steroid receptor pathways. We are ready to move forward and look at the importance of the estrogen receptors in the mechanisms of genistein and resveratrol. We also look forward to determining the effect of these polyphenols in a novel mouse model for mammary cancer, one which over-expresses AIB1, and critical sex steroid coactivator in the mammary gland. There is much more to learn about the mechanisms of these polyphenols so that they may be used in clinical trials and help women to decrease their risk of breast cancer.

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